Ryuso TANAKA* & Chigusa TAKAHASHI*: **Comparative karyotype analysis in *Epimedium* species by C-banding (1) *E. sempervirens* var. *hypoglaucum* and *E. perralderianum*****

田中隆荘*・高橋ちぐさ*: C-バンディングによるイカリソウ属の種の比較核型分析 (1) ウラジロイカリソウと北アフリカ産 *Epimedium perralderianum***

The species of *Epimedium* occur in north temperate regions, mainly Asia and the Mediterranean region. About twenty species are reported in those regions (Stearn 1938). Most of these species are highly polymorphic in external morphology showing taxonomical and phylogenetic complication (Stearn, 1938; Nakai, 1944; Maekawa, 1955; Shimizu, 1960; Ono, 1965; Suzuki, 1978). In contrast to these morphological variations, the chromosome number, $2n=12$, was reported to be the same in 13 species and similar karyotypes in the mitotic metaphase were observed by several workers (Langlet, 1928; Miyaji, 1930; Kurita, 1956; Koyama, 1956; Kuroki, 1967, 1970).

In plants, it is usually known that the morphological variations are well correlated to the chromosomal variations. In *Epimedium*, however, the chromosomes were reported to be uniform although the morphological variations would be seen. For more detailed information about the chromosome morphology, C-banding technique has been applied to the karyotype analysis of many plants and animals in recent years. Several studies on plant species have demonstrated that this method serves as a useful aid to the understanding of the interspecific relationship in plant species (Schweizer and Ehrendorfer, 1976; Vosa, 1976). The authors have carried out C-banding karyotype analysis of the taxa of *Epimedium* in Japan and some taxa in Asia, Europe and north Africa (Takahashi and Tanaka 1979). The present paper is a preliminary report dealing with the karyotype analysis.

Materials and methods Materials used and their localities: *Epimedium sempervirens* Nakai var. *hypoglaucum* (Makino) Ohwi, a native and common species of

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Japan, were collected from Echizen-cho, Fukui Pref., Japan. *E. perralderianum* Cosson, a native species of Algeria in north Africa, were obtained from Dr. R. Weaver in the Arnold Arboretum, U. S. A., to whom we express thanks for the supply of materials. Validating specimens of the clones studied were deposited in the Herbarium of the Botanical Institute of Hiroshima University.

For the cytological preparations root tips were pretreated with 0.002 M 8-hydroxyquinoline for 2.5-3 hours at 18-20°C and fixed in ethanol-glacial acetic acid (3 : 1) overnight at 5°C. For orcein staining the fixed materials were macerated in 45% acetic acid-1N HCl (1 : 2) for 10-15 seconds at 60°C. The macerated materials were stained with 2% aceto-orcein and squashed. For C-banding the procedure of Tanaka and Taniguchi (1975) was applied : The fixed materials were macerated in the same method, rinsed in deionized water and squashed in 45% acetic acid. After the cover slips were removed by dry-ice method, the slides were air-dried for 3-7 days. The air-dried slides were incubated in 5% aqueous solution of $Ba(OH)_2 \cdot 8H_2O$ for 5-7 minutes at 50°C, rinsed in deionized water and then incubated in 2×SSC for 1.5 hours at 60°C and rinsed in deionized water. They were stained in 1.5% Giemsa (Merck Co.) diluted with 1/15 M phosphate buffer at pH 6.8 for about 30 minutes, rinsed in deionized water, air-dried and mounted in Eukitt (Kindler Co.).

Observations Studies on the karyotypes have been carried out by the present authors on 12 taxa of *Epimedium* from Japan, 5 taxa from Asia, Europe and north Africa (Takahashi and Tanaka 1979) and at present it is still under investigation. All of the taxa studied were 2n=12, and among them mitotic metaphase chromosomes were similar in size and in the position of centromere. These verified the previous reports of Langlet (1928), Miyaji (1930), Kurita (1956), Koyama (1965) and Kuroki (1967, 1970).

1. **Epimedium sempervirens** Nakai var. **hypoglaucum** (Makino) Ohwi (Figs. 1A, 2A and 2C)

Five clones observed were found to be 2n=12, which confirmed the previous report of Kuroki (1970). The somatic chromosomes at metaphase varied in length from 7.0 μm to 5.2 μm and could be classified into 6 pairs. All of the chromosomes had median or submedian centromere (Fig. 1A). Three of the pairs (chromosome 1, 2; 3, 4; 5, 6) were metacentric chromosomes which varied in arm ratio ranging from about 1.1 to 1.5. Secondary constrictions were found in the short arms of chromosome 1 and 2. The remaining three pairs (chromo-

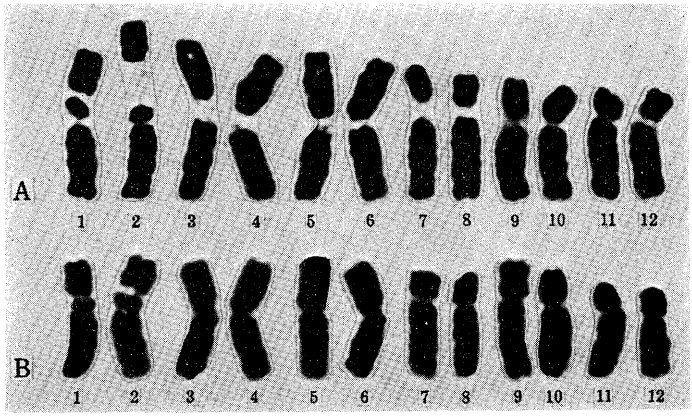

Fig. 1. Photomicrographs of chromosomes at mitotic metaphase in two species of *Epimedium*. A. *E. sempervirens* var. *hypoglaucum* (2n=12). B. *E. perralderianum* (2n=12). ×2600.

some 7, 8; 9, 10; 11, 12) were submetacentric chromosomes which varied in arm ratio ranging from about 2.0 to 2.6. Two of these pairs (chromosome 7, 8; 9, 10) were similar in size, but the short arms of chromosome 7 and 8 were shorter than those of chromosome 9 and 10. The pair of chromosome 11 and 12 was the shortest of the complement.

All of the somatic chromosomes of *E. sempervirens* var. *hypoglaucum* possessed C bands (Fig. 2A). The idiogram for the banding patterns of this species is shown in Fig. 2C.

In the pair of chromosome 1 and 2, two large and three small bands were observed (Figs. 2A-1, -2 and 2C-1, -2). One of the large bands was located in the region adjacent to the secondary constriction on the satellite and the other in the interstitial region of the long arm, and the small bands were located in the centromeric region and in the terminal and interstitial region of the long arm.

In the pair of chromosome 3 and 4, five small bands were observed (Figs. 2A-3, -4 and 2C-3, -4). One was located in the centromeric region, one terminally in the short arm, one terminally in the long arm, one in the distal fourth of the short arm and one in the distal fourth of the long arm.

In the pair of chromosome 5 and 6, three small bands were observed (Figs. 2A-5, -6 and 2C-5, -6). One was located in the centromeric region, one terminally in the short arm and one terminally in the long arm.

In the pair of chromosome 7 and 8, three medium-sized and two small bands were observed (Figs. 2A-7, -8 and 2C-7, -8). All of the medium-sized bands were located interstitially in the long arm, and the small bands in the centromeric region and terminally in the long arm.

In the pair of chromosome 9 and 10, four small bands were observed (Figs. 2A-9, -10 and 2C-9, -10). One was located in the centromeric region, one

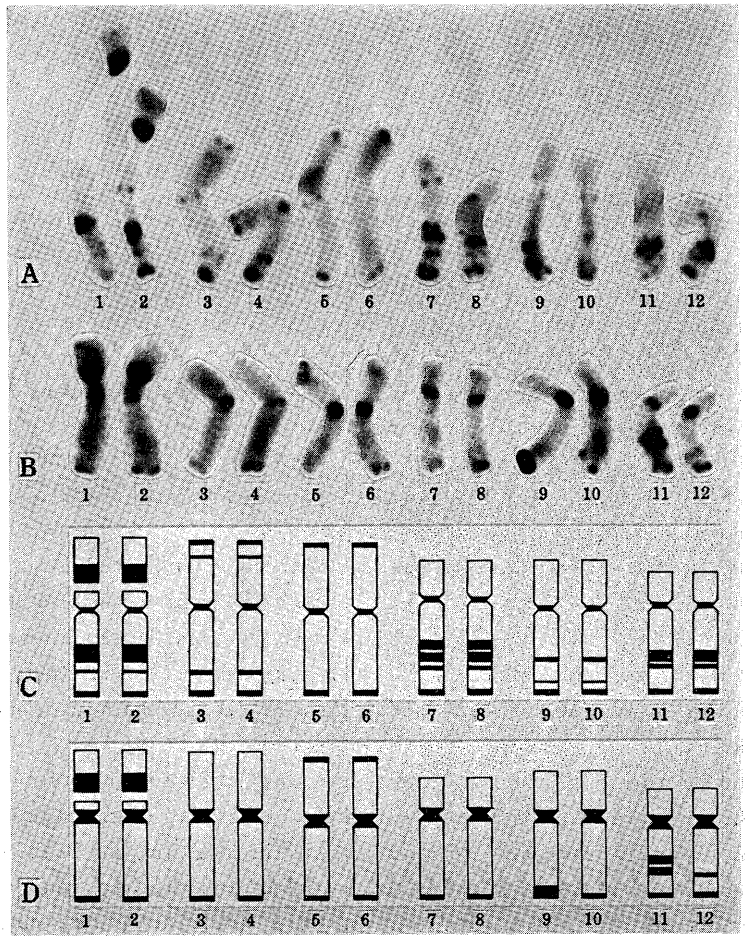

Fig. 2. Photomicrographs of C-banded chromosomes at mitotic metaphase and idiogramatic representations in two species of *Epimedium*. A, C. *E. sempervirens* var. *hypoglaucum* (2n=12). B, D. *E. perralderianum* (2n=12). ×2600.

terminally and two interstitially in the long arm.

In the pair of chromosome 11 and 12, two medium-sized and two small bands were observed (Figs. 2A-11, -12 and 2C-11, -12). The medium-sized bands were located interstitially in the long arm, and the small bands in the centromeric region and terminally in the long arm.

As described above, C bands were observed in the centromeric, terminal and interstitial regions and in the region adjacent to the secondary constriction on the satellite. Centromeric bands were observed to be small in size in all of the chromosomes. Terminal bands were also small in size, and appeared in both arms of chromosome 3-6 and in the long arms of chromosome 1, 2 and 7-12. Interstitial bands varied in size and were present in both arms of chromosome 3 and 4 and in the long arms of chromosome 1, 2 and 7-12. The bands on the satellites were large-sized.

2. **E. perralderianum** Cosson (Figs. 1B, 2B and 2D)

Two clones investigated were found to be 2n=12. The present data is the first report of karyotype analysis in *E. perralderianum*. The somatic chromosomes at metaphase varied in length from 6.8 μm to 5.0 μm and could be classified into 6 pairs. All of the chromosomes had median or submedian centromere (Fig. 1B). Three of the pairs (chromosome 1, 2; 3, 4; 5, 6) were metacentric chromosomes which varied in arm ratio ranging from about 1.1 to 1.5. Secondary constrictions were found in the short arms of chromosome 1 and 2. The remaining three pairs (chromosome 7, 8; 9, 10; 11, 12) were submetacentric chromosomes which varied in arm ratio ranging from about 1.8 to 2.9. The karyotype of this species is similar to that of the previous *E. sempervirens* var. *hypoglaucum* except that chromosome 9 and 10 were somewhat larger than chromosome 7 and 8 in length.

C bands were observed in all of the somatic chromosomes of *E. perralderianum* (Fig. 2B). The idiogram for the banding patterns of this species is shown in Fig. 2D.

In the pair of chromosome 1 and 2, one large one medium-sized and one small bands were observed (Figs. 2B-1, -2 and 2D-1, -2). The large band was located in the region adjacent to the secondary constriction on the satellite, the medium-sized band in the centromeric region, and the small band terminally in the long arm.

In the pair of chromosome 3 and 4, one medium-sized and one small bands

were observed (Figs. 2B-3, -4 and 2D-3, -4). The medium-sized band was located in the centromeric region, and the small band terminally in the long arm.

In the pair of chromosome 5 and 6, one medium-sized and two small bands were observed (Figs. 2B-5, -6 and 2D-5, -6). The medium-sized band was located in the centromeric region, and the small bands terminally in both arms.

In the pair of chromosome 7 and 8, one medium-sized and one small bands were observed (Figs. 2B-7, -8 and 2D-7, -8). The medium-sized band was located in the centromeric region, and the small band terminally in the long arm.

In the pair of chromosome 9 and 10, chromosome 9 possessed one large band terminally in the long arm and one medium-sized band in the centromeric region, but chromosome 10 possessed one small band terminally in the long arm and one medium-sized band in the centromeric region (Figs. 2B-9, -10 and 2D-9, -10). Thus, this pair was heteromorphic with respect to the size of the terminal band.

In the pair of chromosome 11 and 12, chromosome 11 possessed two medium-sized band interstitially in the long arm, one medium-sized band in the centromeric region and one small band terminally in the long arm, but chromosome 12 possessed only one small band interstitially in the long arm, one medium-sized band in the centromeric region and one small band terminally in the long arm (Figs. 2B-11, -12 and 2D-11, -12). Thus, this pair was heteromorphic with respect to the size and number of the interstitial band.

As described above, centromeric bands were observed to be medium-sized and in all of the chromosomes. Terminal bands were small or large in size, and appeared in both arms of chromosome 5 and 6 in the long arms of chromosome 1-4 and 7-12. Interstitial bands were found only in the long arms of chromosome 11 and 12. The bands on the satellites were large-sized. Thus, the C-banding patterns of this species were different from those of the previous species, *E. sempervirens* var. *hypoglaucum*. That is, the number of interstitial bands was fewer than that of previous species, and all of the chromosomes possessed distinct centromeric bands which were larger than those of previous species.

The north African species *E. perralderianum* was found to show the different C-banding patterns from the Japanese species, *E. sempervirens* var. *hypoglaucum*.

Summary

1. Karyomorphological studies were carried out in *Epimedium sempervirens* var. *hypoglaucum* (2n=12) from Japan, and *E. perralderianum* Cosson (2n=12) from north Africa.

2. The karyotypes of the two species closely resembled each other in chromosome size and in the position of the centromere. The chromosome complement consisted of three pairs of submetacentric chromosomes and three pairs of metacentric chromosomes, one of which had a secondary constriction at the proximal region of the short arm.

3. The C-banding patterns of *E. sempervirens* var. *hypoglaucum* and *E. perralderianum* were found to differ considerably. In *E. perralderianum* interstitial bands were fewer and centromeric bands were larger than those of *E. sempervirens* var. *hypoglaucum*.

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Epimedium L. イカリソウ属の種は，日本産でも，また外国産の間でも，分裂期中期染色体の核型が同一であって (Langlet 1928, Koyama 1965, ほか)，核型は外部形態の複雑な変異と対応していないことが報告されている。著者らは，本属の種のうち日本産の種と外国産の種の間で，Cバンド模様に大きな相違があることを見出した（高橋・田中 1979)。本報告は，その結果の一部である。すなわち，日本産の *E. sempervirens* var. *hypoglaucum*, ウラジロイカリソウ (2n＝12) では，中部動原体型染色体の 1 対を除き，他のすべての染色体に介在バンドが観察された。一方，アルジェリア産の *E. perralderianum*, (2n＝12) では，介在バンドは，最小の染色体 1 対のみに観察された。また，動原体部のバンドは，両種ともに，すべての染色体に観察されたが，その大きさが，ウラジロイカリソウでは小さく，*E. perralderianum* では，ウラジロイカリソウのそれらの二倍以上の大きさであった。

□池原直樹著，多和田真淳監修：**沖縄植物野外活用図鑑**　全 6 巻. 1979. 新星図書出版. ¥19,800. 宜野座高校教諭の著者が撮影したカラースライドを監修者がすべて同定し，著者が方言名，撮影年月，場所および解説をつけたものである。1. 栽培植物と果樹，2. 栽培植物，3. 帰化植物，4. 海辺の植物とシダ，5. 低地の植物，6. 山地の植物，の 6 巻に約 1460種が収載されている。写真，印刷とも見事なできばえで美しい。なかなかお目にかかれない沖縄植物の写真図鑑として推せんに値する。新和名もいくつか発表されている。全体の索引があったらなお便利だろう。　（金井弘夫）